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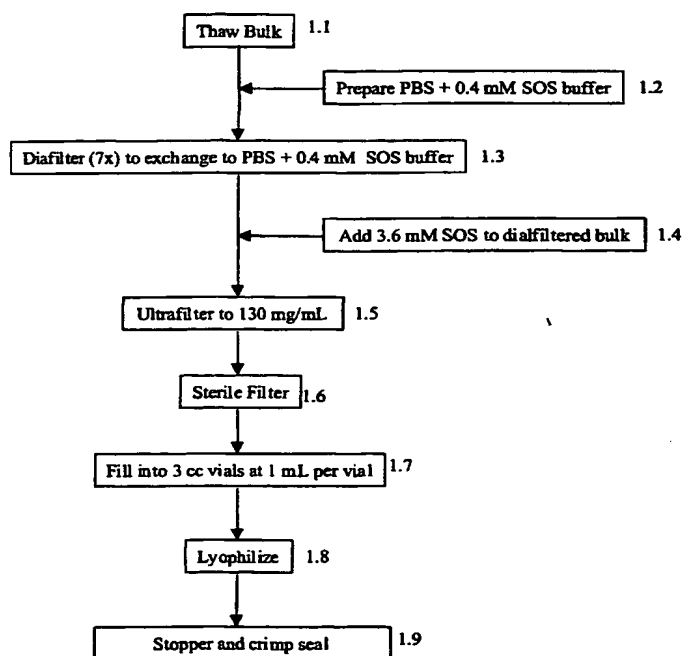
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[Continued on next page]

(54) Title: METHOD OF PRODUCING AND PURIFYING ENDOSTATINTM PROTEIN

Process Flow Diagram for Formulation/Fill/Finish
Formulation I: rhEndostatin/SOS



(57) Abstract: The present invention provides a method for recombinant production, recovery and purification of EndostatinTM protein. This method may be employed for large scale recovery and purification of recombinantly-produced EndostatinTM protein.

Note: The lyophilized product will be reconstituted with commercially available bottled Water For Injection (WFI)



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(81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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9 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 05213-0550P	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 00/ 25166	International filing date (day/month/year) 14/09/2000	(Earliest) Priority Date (day/month/year) 14/09/1999
Applicant ENTREMED, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1
☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/25166

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/78 C12P21/02 C12N1/16 C07K1/16
 //(C12P21/02,C12R1:84)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, CHEM ABS Data, EMBL, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 26480 A (GENETIX PHARMACEUTICALS INC ;MASSACHUSETTS INST TECHNOLOGY (US)) 3 June 1999 (1999-06-03) SEQ ID NO: 50 SEQ ID NO: 76 examples 1-4	1,5-7
X	JOHN HARALD ET AL: "Novel glycosylated forms of human plasma endostatin and circulating endostatin-related fragments of collagen XV." BIOCHEMISTRY, vol. 38, no. 32, 10 August 1999 (1999-08-10), pages 10217-10224, XP002162137 ISSN: 0006-2960 page 10218; figure 1	1-8

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

6 March 2001

Date of mailing of the international search report

19/03/2001

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/25166

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DHANABAL ET AL: "Endostatin: Yeast production, mutants, and antitumor effect in renal cell carcinoma" CANCER RESEARCH,US,AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 59, no. 1, 1999, pages 189-197, XP002100110 ISSN: 0008-5472 page 190 ---	1,3-10
X	BOEHM ET AL: "Zinc-binding of endostatin is essential for its antiangiogenic activity" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,US,ACADEMIC PRESS INC. ORLANDO, FL, vol. 252, 1998, pages 190-194, XP002100108 ISSN: 0006-291X page 191, left-hand column ---	1,3-10
X	T BOEHM ET AL: "Disruption of the KEX1 gene in Pichia pastoris allows expression of full-length murine and human endostatin" YEAST,CHICHESTER, SUSSEX,GB, vol. 15, 1999, pages 563-572, XP002109421 ISSN: 0749-503X page 564, right-hand column -page 565, left-hand column ---	1,3-10
P,X	SHEPARD SCOT ET AL: "Purification of recombinant human Endostatin and Angiostatin from Pichia pastoris fermentation broth: Expanded Bed Adsorption chromatography method development and scale up." ABSTRACTS OF PAPERS AMERICAN CHEMICAL SOCIETY, vol. 219, no. 1-2, 2000, page BIOT 212 XP000982564 219th Meeting of the American Chemical Society.;San Francisco, California, USA; March 26-30, 2000 ISSN: 0065-7727 the whole document ---	1,3-8
A	WO 96 08274 A (PRIZM PHARMA INC) 21 March 1996 (1996-03-21) page 61, line 5 - line 29; example 11 page 25, line 10 - line 20 --- -/--	11-16

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/25166

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>STANDKER LUDGER ET AL: "Isolation and characterization of the circulating form of human endostatin."</p> <p>FEBS LETTERS, vol. 420, no. 2-3, 29 December 1997 (1997-12-29), pages 129-133, XP002161535 ISSN: 0014-5793</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9926480	A	03-06-1999	AU	1598599 A	15-06-1999
WO 9608274	A	21-03-1996	AU	3724495 A	29-03-1996
			US	6037329 A	14-03-2000
			AU	3374795 A	22-03-1996
			AU	710309 B	16-09-1999
			AU	5862896 A	29-11-1996
			CA	2221269 A	21-11-1996
			EP	0833665 A	08-04-1998
			JP	11505805 T	25-05-1999
			WO	9606641 A	07-03-1996
			WO	9636362 A	21-11-1996